

Claims.

What is claimed is:

1. A method of isolating extrachromosomal nucleic acids in a substantially purified
5 form, the method comprising the steps of:
 - a) homogenizing a biological sample;
 - b) removing substantially all of the chromosomal nucleic acids from the biological
sample;
 - c) contacting the biological sample containing extra-chromosomal nucleic acids
10 with an effective amount of a chaotropic solution under conditions which permit the
nucleic acids in the supernatant to precipitate; and
 - d) recovering the extrachromosomal nucleic acids.
2. The method of claim 1, further comprising the step, prior to step c), of contacting
15 the biological sample with a ribonuclease enzyme under conditions sufficient to
subject the sample to endonucleolytic cleavage of RNA.
3. The method of claim 2, further comprising the step, prior to step c), of precipitating
said extrachromosomal nucleic acids and resuspending the nucleic acids in buffer.
20
4. The method of claim 3, wherein the homogenizing of the biological sample is by
incomplete lysis.
5. The method of claim 4, wherein the incomplete lysis is by alkaline lysis.

6. The method of claim 5, wherein the chromosomal nucleic acids are removed by the method selected from the group consisting of precipitation, sedimentation, filtration, and centrifugation.
- 5
7. The method of claim 6, further comprising the step of washing said recovered extrachromosomal nucleic acids
8. The method of claim 7, wherein the washing is by addition of an alcohol and water
- 10 solution.
9. The method of claim 6, wherein the biological sample is a cell system selected from the group consisting of bacteria, yeast, insect, plant and mammalian.
- 15 10. The method of claim 9, wherein the cell system contains one or more extracellular nucleic acid sources selected from the group consisting of recombinant bacteriophage, plasmid, cosmid, yeast expression vector, viral expression vector, and retrovirus.
- 20 11. The method of claim 5, wherein the recovery of said nucleic acids from said solution is by adding an effective amount of organic solvent thereto, and recovering the precipitated nucleic acids.
12. The method of claim 11, wherein said organic solvent is selected from the group

consisting of lower alcohols, acetone, polyethylene glycol and dimethylsulfoxide.

13. The method of claim 6, wherein said chaotropic solution comprises:

a) effective amounts of at least one chaotropic agent, and

5 b) a buffer present in an amount sufficient to maintain said solution at an alkaline pH.

14. The method of claim 6, wherein said chaotropic agent is selected from the group consisting of guanidine thiocyanate, sodium iodide, sodium perchlorate, guanidine
10 hydrochloride, urea, sodium hydroxide, potassium hydroxide, guanidine salt, potassium thiocyanate, formamide, and sodium chloride, sodium iodide and mixtures thereof.

15. The method of claim 14, wherein said chaotropic agent is present at a
15 concentration in the range of about 1M-7M.

16. The method of claim 14, wherein said chaotropic agent is present at a concentration in the range of about 2M-5M.

20 17. The method of claim 16, wherein said buffer is selected from the group consisting of sodium dihydrogen phosphate and disodium monohydrogen phosphate; sodium dihydrogen phosphate, disodium monohydrogen phosphate and sodium chloride; sodium carbonate, sodium monohydrogen carbonate and sodium chloride; potassium dihydrogen phosphate and sodium monohydrogen phosphate; potassium dihydrogen

phosphate and sodium monohydrogen phosphate; potassium hydrogen tartrate and potassium dihydrogen phosphate; acetic acid and sodium acetate, citric acid and sodium hydroxide; potassium hydrogen phthalate and sodium hydroxide; potassium hydrogen phosphate and sodium phosphate; tris(hydroxymethyl) aminomethane and
5 hydrochloric acid; sodium tetraborate and hydrochloric acid; glycine and hydrochloric acid; triethanolamine and hydrochloric acid; N-tris (hydroxymethyl)methyl-2-amino sulfonic acid and sodium hydroxide; sodium phosphate monobasic, sodium phosphate dibasic, potassium hydrogen tartrate, potassium dihydrogen citrate, potassium hydrogen phthalate, sodium tetraborate, sodium carbonate, sodium hydrogen
10 carbonate and mixtures thereof.

18. The method of claim 17, wherein said buffer is present in an amount sufficient to maintain the pH of said solution in the range of about 7 to about 12.

15 19. The method of claim 17, wherein said buffer is present in an amount sufficient to maintain the pH of said solution in the range of about 7 to about 10.

20. The method of claim 17, wherein said buffer is present in an amount sufficient to maintain the pH of said solution in the range of about 7 to about 8.

20

21. The method of claim 17, wherein said chaotropic solution further comprising one or more compounds selected from the group consisting of solvents, surfactants, detergents, salts, chelating agents, preservatives, anti-oxidants, bacteriostats, solutes, reducing agents, stabilizers and mixtures thereof

22. The method of claim 21, wherein said reducing agent is selected from the group consisting of dithiothreitol (DTT), dithioerythritol (DTE), beta-mercaptoethanol (BME), cysteine, cysteamine, thioglycolate, glutathione, sodium borohydride and
5 mixtures thereof.
23. The method of claim 21, wherein said reducing agent is beta-mercaptoethanol.
24. The method of claim 21, wherein said salt is selected from the group consisting of
10 sodium chloride, potassium chloride, ammonium chloride, sodium acetate, sodium nitrate, lithium chloride, sodium bromide and mixtures thereof.
25. The method of claim 24, wherein said salt is present in the range from about 0.01 M to about 1 M. and preferably in the range of about 0.1 M to about 0.5 M.
15
26. The method of claim 24, wherein said salt is present in the range from about 0.1 M to about 0.5 M.
27. The method of claim 21, wherein said chelating agent is selected from the group
20 consisting of ethylenediamine tetraacetic acid, citric acid and mixtures thereof.
28. The method of claim 27, wherein said chelating agent is present in the range of about 0.05% to about 0.50%.

29. The method of claim 21, wherein said detergent is selected from the group consisting of sorbitan trioleate, sorbitan tristearate, propylene glycol monostearate; sorbitan sesquiolate; glycerol monostearate; sorbitan monooleate; propylene glycol monolaurate; sorbitan monostearate; diethylene glycol monostearate; glycerol monostearate; diethylmonolaurate; sorbitan monopalmitate; sorbitan monolaurate; TRITONs; polyoxyethylene ethers; polyoxyethylene lauryl ether; polyoxyethylene sorbitan monostearate; polyoxyethylene sorbitan monooleate; polyoxyethylene sorbitan tristearate; polyoxyethylene sorbitan trioleate; polyoxyethylene glycol monooleate; polyoxyethylene glycol monostearate; triethanolamine oleate; polyoxyethylene monyl phenol; polyethylene glycol monolaurate; polyoxyethylene sorbitan monolaurate; polyoxyethylene sorbitan monostearate; polyoxyethylene sorbitan monooleate; polyoxyethylene stearyl ether; polyoxyethylene oleyl ether; polyoxyethylene sorbitan monopalmitate; polyoxyethylene cetyl ether; polyoxyethylene stearate; sodium oleate; potassium oleate; cetyl ethyl morpholinium ethosulfate; sodium lauryl sulfate; sodium caprylate; sodium caprate; sodium laurate; sodium myristate; sodium cholate; sodium desoxycholate; sodium dihydrocholate; tetradecyltrimethyl ammonium bromide; hexadecylpyridinium chloride; Tween 20; Tween 30; Tween 80, and mixtures thereof.
30. The method of claim 29, wherein said detergent is present in the range of about 0.001% to about 7.5%.
31. The method of claim 29, wherein said detergent is TRITON X-114®.

32. The method of claim 21, wherein said solvent is selected from the group consisting of lower alcohols, acetone, polyethylene glycol, dimethylsulfoxide, and mixtures thereof..
- 5 33. The method of claim 32, wherein said lower alcohol is selected from the group consisting of methanol, ethanol, iso-propanol, n-propanol, t-butanol, glycerol, propylene glycol, ethylene glycol, polypropylene glycol, polyethylene glycol, and mixtures thereof.
- 10 34. The method of claim 32, wherein said solvent is present in the range of from about 5% to about 40% by volume.
35. The method of claim 32, wherein said solvent is present in the range of from about 15% to about 20% by volume.
- 15 36. The method of claim 35, wherein said solvent is iso-propanol.
37. The method of claim 21, wherein said antioxidant is selected from the group consisting of BHA, BHT, octyl gallate, dodecyl gallate, sodium sulfite, sodium thiosulfite, lactic acid, citric acid, tartaric acid, vitamin C, vitamin E, uric acid and the salts and mixtures thereof.
- 20 38. A method of isolating nucleic acids in a substantially purified form, said method comprising the steps of:

- a) homogenizing a biological sample containing nucleic acids of interest;
- b) partially purifying the nucleic acids from the biological sample by adhering the nucleic acids of interest to a retaining means;
- c) contacting the gel containing the sample with an effective amount of a chaotropic solution under conditions which permit the nucleic acids to precipitate; and
- d) recovering the nucleic acids.

39. The method of claim 38, wherein the retaining means is a solid support matrix

40. The method of claim 39, wherein the solid support matrix is an electrophoresis gel

41. The method of claim 39, wherein the gel is agarose

42. The method of claim 39, wherein the gel is acrylamide

43. The method of claim 40, wherein the region of the gel containing the DNA of interest is excised and solublized prior to contacting the sample with a chaotropic solution.

44. The method of claim 41, wherein the gel is solublized using an enzyme that specifically cleaves the gel matrix bonds without damaging nucleic acids.

45. The method of claim 44, wherein the enzyme is beta-agarose.

46. The method of claim 40, wherein the recovery of said nucleic acids from said solution is by adding an effective amount of organic solvent thereto, and recovering the precipitated nucleic acids.

5

47. The method of claim 46, wherein said organic solvent is selected from the group consisting of lower alcohols, acetone, polyethylene glycol and dimethylsulfoxide.

48. The method of claim 47, wherein said chaotropic solution comprises:

- 10 a) effective amounts of at least one chaotropic agent, and
 b) a buffer present in an amount sufficient to maintain said solution at an alkaline pH.

49. The method of claim 48, wherein said chaotropic agent is selected from the group
15 consisting of guanidine thiocyanate, sodium iodide, sodium perchlorate, guanidine hydrochloride, urea, sodium hydroxide, potassium hydroxide, guanidine salt, potassium thiocyanate, formamide, and sodium chloride, sodium iodide and mixtures thereof.

20 50. The method of claim 49, wherein said chaotropic agent is present at a concentration in the range of about 1M-7M.

51. The method of claim 49, wherein said chaotropic agent is present at a concentration in the range of about 2M-5M.